

BODY COMPOSITION ANALYSIS

IN U.S. NAVY DIVERS

David D. Gummin, M.D.
Toxikon Consortium
1900 W. Polk St., Suite 500
Chicago, IL 60612
W: (312) 633-5310
Fax: (312) 633-6442
dgummin@UIC.edu

REPORT DOCUMENTATION PAGE			<i>Form Approved</i> <i>OMB No. 0704-0188</i>	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of the collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302 and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave Blank)		2. REPORT DATE March 2001	3. REPORT TYPE AND DATES COVERED Technical 6/92 – 5/93	
4. TITLE AND SUBTITLE Body composition analysis in U.S. Navy divers.			5. FUNDING NUMBERS PE – 63713N PR – M0099 TA – 01A/01B WU – 1003/1428	
6. AUTHOR(S) Gummin, D.D.*				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) NAVAL MEDICAL RESEARCH CENTER 503 ROBERT GRANT AVENUE SILVER SPRING, MARYLAND 20910-7500			8. PERFORMING ORGANIZATION REPORT NUMBER NMRC 01-001	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) BUREAU OF MEDICINE AND SURGERY (MED-02) 2300 E STREET, NW WASHINGTON, DC 20372-5300			10. SPONSORING/MONITORING AGENCY REPORT NUMBER DN377011/DN244515	
11. SUPPLEMENTARY NOTES *Current Address: Infinity HealthCare, Inc. and The Wisconsin Poison Center, Children's Hospital of Wisconsin, 9000 W. Wisconsin Ave., Milwaukee, WI 53201				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Physical readiness and body makeup are considered fundamental attributes of U.S. Navy divers. Methods to objectively determine body makeup are fraught with shortcomings and can be technically challenging, particularly in field operations. Two potential field methods of determining body composition, densitometry and deuterium oxide dilution, were assessed and compared in the laboratory. Ten healthy male U.S. Navy divers volunteered for testing by both techniques. Body composition data were then compared between methods and to published data. Significant variability was found in measurements derived by the deuterium oxide dilution method (mean coefficient of variation 4.6%). Normalizing this method to densitometry by linear regression demonstrates a theoretical hydration "constant" for lean body mass of 0.833 ± 0.009 . Published values for this "constant" are typically cited as 0.712, but without substantive experimental support. Deuterium oxide dilution is an insensitive technique for determining small changes in body composition. Published anthropometric data are unreliable if not specifically analyzed and interpreted in the context of the population studied.				
14. SUBJECT TERMS body composition, field methods, densitometry, deuterium oxide dilutions, total body water			15. NUMBER OF PAGES 27	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unclassified	

NSN 7540-01-280-5500

Standard Form 298 (Rev 2-89)
Prescribed by ANSI S10 239-98
298-102

Physical readiness and body makeup are considered fundamental attributes of U.S. Navy divers. Methods to objectively determine body makeup are fraught with shortcomings and can be technically challenging, particularly in field operations. Two potential field methods of determining body composition, densitometry and deuterium oxide dilution, were assessed and compared in the laboratory. Ten healthy male U.S. Navy divers volunteered for testing by both techniques. Body composition data were then compared between methods and to published data. Significant variability was found in measurements derived by the deuterium oxide dilution method (mean coefficient of variation 4.6%). Normalizing this method to densitometry by linear regression demonstrates a theoretical hydration “constant” for lean body mass of 0.833 ± 0.009 . Published values for this “constant” are typically cited as 0.712, but without substantive experimental support. Deuterium oxide dilution is an insensitive technique for determining small changes in body composition. Published anthropometric data are unreliable if not specifically analyzed and interpreted in the context of the population studied.

TABLE OF CONTENTS

	page
Acknowledgements.....	iii
Background.....	1
Methods	2
Densitometry.....	2
Total body water	4
Assays	5
Analyses.....	6
Results.....	6
Body Mass Determination	6
Densitometry.....	7
Lean Body Mass	8
Deuterium Oxide Dilution	9
Summary	16
References.....	21

List of Tables

Table 1. Mean Body Mass	6
Table 2. Hydrostatic Weights	8
Table 3. Lean Body Mass Calculated from Densitometry.....	9
Table 4. Total Body Water Calculated from Plasma Deuterium Oxide Dilution.....	9
Table 5. Total Body Water Calculated from Urinary Deuterium Oxide	10
Table 6. Variation in Total Body Water from Plasma Deuterium Oxide Dilution	11
Table 7. Variation in Total Body Water from Urinary Deuterium Oxide	11
Table 8. Pearson Correlation Analysis of Variance using Urinary Deuterium Oxide	12

List of Figures

Figure 1. Subject Weight vs. TBW	13
Figure 2. Density-determined Lean Mass vs. TBW	15

ACKNOWLEDGEMENTS

The author expresses gratitude to the divers of Naval Medical Research Institute Diving Medicine Department who participated in the study. Special thanks to HMC James Mancuso and the other technicians in the Hyperbaric Environmental Adaptation Program for their extensive technical support. This research was partially supported by the Naval Medical Research and Development Command work units 63713N M0099-01A.1003 and 63713N M0099-01B.1428. The opinions and assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

The research protocol employing human subjects in this study has been reviewed and approved by the Naval Medical Research Institute's Committee for the Protection of Human Subjects.

BACKGROUND

Body composition is an essential feature in evaluating an individual's fitness to dive, as evidenced by the prolific history of projects dedicated to this issue (1-22). Fitness-to-dive qualifications include adherence to the physical readiness and body fat standards stated in the Manual of the Medical Department (23). The readiness standards set down in the naval instruction (24) were recently updated (25) though the body composition criteria for personnel readiness remain essentially unchanged.

More essential to undersea medicine is an appreciation of the role of individual body tissues in systemic physiologic processes. These include metabolic activity; pharmacologic distribution and processing; nutritional and hydrational regulation; distribution, absorption and retention of inert gas burdens; and the degree of involvement of each tissue in each of these processes. Body composition has long been purported to affect an individual's ability to decompress safely from dives or submarine escape scenarios (8-10,14,19). Human decompression experience has not borne this out, nor is there strong epidemiologic support for this concept (7,22).

To assess currently available indices of body composition in U.S. Navy divers, two analytical techniques were evaluated: deuterium oxide (D₂O) dilution and hydrostatic weighing (or densitometry). Healthy divers at the Naval Medical Research Institute (NMRI) were solicited to undergo body composition analysis by these two widely accepted techniques.

METHODS

Ten healthy male U.S. Navy diver volunteers aged 22-44 ate a standardized diet for 24 h prior to each day of the study. All subjects had a current diving medical examination on file in their medical record, were free of cardiovascular, pulmonary or renal disease, and were qualified to participate by the Head of the Health Monitoring Division at NMRI. The diet was a typical mixed American diet that provided approximately 3300 Cal, 3.5 gm of sodium, and 4.4 gm of potassium. Caloric intake consisted of 15% protein, 30% fat, and 55% carbohydrates. Although fluid intake was *ad libitum* over the 24 h preceding the day of the study, additional calories, caffeine, and alcohol intake were not permitted. All subjects were hydrostatically weighed to calculate body density in the manner described by Brozek et al. (3).

Densitometry

While immersed, each subject's pulmonary residual volume was determined by taking the mean of two measurements of oxygen-dilution as described by Wilmore et al.²¹:

$$RV = VO_2 \cdot \frac{(b - a)}{(c - d)} \quad [\text{Eq. 1}]$$

where, RV = pulmonary residual volume in ml; VO_2 = volume of O_2 in the bag at the beginning of the procedure; a = % N_2 impurity in the original O_2 breathed; b = % N_2 in the mixed gas in the bag at equilibrium (= 100% - % O_2 - % CO_2); c = % N_2 in the alveolar air at the beginning of the test; and d = % N_2 in the alveolar air during the last maximal breath (= b + 0.2% N_2).

Simplified, this becomes

$$RV = \frac{VO_2 \cdot b}{79.8 - b} \quad [\text{Eq. 2}]$$

To then determine body density, accounting for lung residual volume, subjects were hydrostatically weighed. Taking the standard equation for body density from Brozek (3) adjusting for residual volume, and correcting for water density at the temperature of the test (35.5 °C):

$$D_B = \frac{BM}{\frac{BM - \text{Wet wt}}{(D_w)} - RV} \quad [\text{Eq. 3}]$$

where D_B = Body density in gm/cm^3 ; BM = body mass of the subject in air; Wet wt = weight of the subject weighed when immersed; RV = lung residual volume in ml; and D_w = density of water in the tank at the temperature of the test.

Body density was then used to calculate percent body fat as:

$$\% BF = \frac{4.570^*}{D_B} - 4.142^* \cdot 100 \quad [\text{Eq. 4}]$$

with * = Constants from Brozek. Lean body mass was then calculated as:

$$LBM = BM - \frac{BM \cdot \%BF}{(100)} \quad [\text{Eq. 5}]$$

Total Body Water

Total body water (TBW) was determined by D₂O dilution on 5 individual days, each following a day of dietary control. Determination of TBW content was performed for both plasma and urine samples. Because one subject was unable to participate in the last trial of the study and several others gained weight before the last trial, the fifth trial of the data set was excluded from analyses of TBW determinations.

On the morning of each day of the study, subjects first underwent a 2-hour prehydration period. During that period, each drank 2.5 ml/kg body weight of deionized water every 30 min. Thirty minutes following the last water consumption, subjects drank a 10-gram (gravimetrically weighed) dose of deuterated water (Deuterium Oxide, 99.9% enriched, Cambridge Isotopes, Woburn, MA). The cup was rinsed 3 times with a total of 100 cc of deionized water. A 2-hour equilibration period ensued during which subjects were seated upright and at rest. Following D₂O equilibration, and precisely 4 h after the hydration period began, plasma and urine specimens were obtained for ²H/H ratio analysis. Schoeller and colleagues (15) allowed a 6-hour equilibration period, but found that H₂¹⁸O equilibrated completely within 1-2 h for healthy subjects. This is consistent with previous findings for deuterium oxide (6).

Individual test days for the four TBW assays were over the course of a 48-day period, during which the subjects participated in a larger, more comprehensive hydration study. To ensure that no ordering or work-up effect would be present in that study, no fewer than 7 days

intervened between test days. No subject underwent hyperbaric or exercise stress in the 24 h preceding body composition analysis. All subjects were counselled on the possible risks and benefits of participation in this study, after which each subject gave written, witnessed informed consent to participate.

Assays

Total body water was determined by correcting D₂O dilution space. Two hours after drinking a 10-gram dose of D₂O, serum and urine specimens were collected. These were submitted to a commercial laboratory (Metabolic Solutions, Inc., Acton, MA) for isotope ratio mass spectrometry. ²H/H ratio was determined in the reference standard, Acton tap water, and transposed to a ratio against Vienna Standard Mean Ocean Water (VSMOW). Total body water was calculated as follows (from Schoeller, et al., who cite a 1% coefficient of variation with this method (15)):

$$TBW = \frac{d}{mw} \cdot \frac{APE}{100} \cdot 18.02 \cdot \frac{1}{R_{std} \cdot \Delta\sigma^2 H} (kg) \quad [\text{Eq. 6}]$$

where, d = the dose of D₂O in grams, mw = the molecular weight of deuterium oxide, APE = percent enrichment deuterium, R_{std} = the ratio of ²H/H in VSMOW (= 0.00015576), and Δδ²H = the difference between baseline and equilibrium deuterium enrichment in the samples. To correct for the 4% overestimation of TBW by the deuterium method, values calculated from the D₂O analysis were divided by 1.04 to get the reported values, as has been suggested from the literature (5,13,20).

Analyses

Systat, Version 5.0 for Windows™ (Systat, Inc., Evanston, IL) was used for all statistical analyses. Data were analyzed by one-way and two-way repeated measures analysis of variance (ANOVA). Where significant changes were detected, a Newman-Keuls analysis was performed to identify those differences.

Subject	Table 1. Mean Body Mass (kg)				
	<i>Trial A</i>	<i>Trial B</i>	<i>Trial C</i>	<i>Trial D</i>	<i>Trial E</i>
1	95.68	94.78	95.44	95.78	96.14
2	72.28	71.86	72.72	72.38	72.86
3	80.48	81.38	80.32	80.00	80.58
4	83.20	83.54	83.50	84.18	84.36
5	93.78	93.52	94.02	94.66	94.12
6	69.50	69.38	70.38	70.20	71.00
7	104.02	103.84	103.84	104.40	
8	71.88	71.92	71.77	71.64	71.32
9	80.08	81.20	81.36	80.90	82.24
10	81.88	81.80	82.28	83.12	83.66

$p=0.007$, $F=4.333$, $df=4,32$

RESULTS

Body mass determination

Subjects were weighed daily, after the hydration period, and immediately prior to D₂O administration. Those results are shown in Table 1. Mean body mass of 9 subjects in Trial E differed from those of the 9 subjects in the previous trials, ($p=0.007$, $F=4.333$, $df=4,32$). A Newman-Keuls analysis identified Trial E as the sole outlier, which formed the basis for

exclusion of this trial from repeated measures analyses. A pooled estimate of variance was calculated for body mass:

$$\sigma^2_{\text{pooled}} = \sum(n_i-1) \cdot \sigma^2_i \text{ [Eq. 7]}$$

where n = the number of observations for each subject, i = the subject number, σ^2_i = the between-trials variance, and σ^2_{pooled} = the pooled estimate of variance. The pooled variance for Trials A-D was 0.316 kg^2 , giving a pooled standard deviation for these trials of 0.563 kg . The mean of the coefficients of variation (c.v.) was 0.005 .

Densitometry

Table 2 gives the results determined by hydrostatic weighing for the 10 subjects.

Table 2. Densitometry							
<i>Subj</i>	<i>Underwater Body Mass (kg)</i>	<i>Height (cm)</i>	<i>Avg Pulmonary Residual Vol* (ml)</i>	<i>Underwater Weight (gm)</i>	<i>Body Density (gm/cm³)</i>	<i>Body Fat (%)</i>	<i>Lean Body Mass[#] (kg)</i>
1	94.14	180.34	1778	2485	1.04098	24.81	70.79
2	72.32	173.99	1423.5	3180	1.06124	16.43	60.44
3	79.52	170.18	1386.5	2680	1.04719	22.20	61.86
4	83.00	185.42	1354	3865	1.06054	16.70	69.13
5	93.20	187.96	1291	3180	1.04379	23.63	71.18
6	68.70	170.18	1140	4115	1.07601	10.52	61.47
7	103.18	187.96	1961	2650	1.04040	25.05	77.33
8	71.12	179.07	1613.5	2665	1.05727	18.04	58.29
9	79.52	171.45	1192	3140	1.05106	20.60	63.14
10	81.56	180.34	1054.5	3645	1.05450	19.14	65.95

*Average of two independent measures

[#]Calculated from body density

Lean Body Mass

Because variance in total BM was so low across Trials A-D, it was assumed that body composition (i.e., body density) remained constant for each individual in each of these 4 trials. Lean body mass was then calculated from the initial body density and the measured body mass on each day of Trials A-D, using Eqs. 4 and 5. These are tabulated in Table 3.

Table 3. Lean Body Mass (kg) Calculated for Each Trial Day

<i>Subject</i>	<i>Trial A</i>	<i>Trial B</i>	<i>Trial C</i>	<i>Trial D</i>
1	71.94	71.27	71.76	72.02
2	60.40	60.05	60.77	60.49
3	62.61	63.31	62.49	62.24
4	69.31	69.59	69.56	70.12
5	71.62	71.42	71.80	72.29
6	62.19	62.08	62.98	62.81
7	77.96	77.83	77.83	78.25
8	58.91	58.95	58.82	58.72
9	63.58	64.47	64.60	64.23
10	66.21	66.14	66.53	67.21

Deuterium oxide dilution

Using the equation from Schoeller (15) D₂O space was calculated for each subject. Total body water was then calculated by adjusting for the 4% overestimation of the D₂O dilution. Results for TBW determined from plasma D₂O are in Table 4, and for urine-determined TBW, in Table 5. Data were available for all study days from urine for 8 of the subjects, and for 9 subjects in Trials A-D.

Table 4. Total Body Water (kg) Determined from Plasma

<i>Subject</i>	<i>Trial A</i>	<i>Trial B</i>	<i>Trial C</i>	<i>Trial D</i>	<i>Trial E</i>
1	55.64	56.47	52.89	55.58	62.92
2	48.29	50.72	50.45	47.62	48.28
3	49.66	53.18	47.77	48.71	51.37
4	55.32	54.90	54.99	54.43	46.58
5	54.95	49.25	55.44	56.60	57.18
6	48.65	48.45	46.00	49.72	53.33
7	59.88	67.98	59.24	66.21	
8	45.53	54.68	48.10	52.79	44.84
9	53.66	51.33	52.65	46.50	53.49
10	54.38	56.06	62.10	60.72	50.22

$p = 0.548$, $F = 0.722$, $df = 3, 27$

Table 5. Total Body Water (kg) Determined From Urine

<i>Subject</i>	<i>Trial A</i>	<i>Trial B</i>	<i>Trial C</i>	<i>Trial D</i>	<i>Trial E</i>
1	60.77	53.74	54.07	47.15	57.71
2	43.78	48.76	44.07	43.73	44.43
3	58.80	48.26	59.86	48.21	49.53
4	52.50	57.49	59.22	46.48	49.44
5	75.14	68.46	63.90	57.15	88.14
6	50.11	47.05	44.88	45.60	44.20
7	58.43	71.13	43.57	59.88	
8	45.04	53.10	45.83	42.99	43.59
9	42.79	50.35	50.41	46.10	54.32
10	50.94	61.80	53.10		50.02

$p = 0.086$, $F = 2.476$, $df = 3, 24$

Analysis of TBW measurements showed no significant difference between the values determined on each of the trial days. This was true for the values calculated from the plasma samples ($p = 0.548$, $F = 0.722$, $df = 3, 27$) and from the urine samples ($p = 0.086$, $F = 2.476$, $df = 3, 24$). A two-way repeated measures ANOVA demonstrated no difference between the measured TBW values for plasma and urine ($p = 0.892$, $F = 0.019$, $df = 1, 16$). Because urine data was not available for all 4 trials, one subject was excluded from the one-way ANOVA for the urine TBW test, as well as from the two-way ANOVA. The pooled variance for the plasma method was 12.683 kg^2 , while the urine demonstrated a higher pooled variance, 56.852 kg^2 . The mean coefficient of variance (c.v.) for the plasma method was 0.049.

To address possible changes in body composition over the course of the 48 days of the study, the absolute difference between TBW measurements was determined for each individual over the shortest and the longest intervals between assays. Those results are presented in Tables 6 and 7 for both the plasma and the urine determination methods.

Table 6. Interval Changes in TBW (by Plasma Method) between Test Days				
<i>Subject</i>	<i>Shortest Interval (days)</i>	<i>Δ Total Body Water (kg)</i>	<i>Longest Interval (days)</i>	<i>Δ Total Body Water (kg)</i>
1	9	3.58	37	0.06
2	8	0.27	34	0.67
3	8	5.41	35	0.94
4	8	0.09	39	0.89
5	12	5.70	48	1.66
6	10	3.72	36	1.08
7	10	8.73	44	6.33
8	8	6.58	40	7.26
9	8	1.32	35	7.16
10	8	6.03	35	6.34
<i>Mean ± SE</i>		<i>4.14 ± 0.91</i>		<i>3.24 ± 0.97</i>

Table 7. Interval Changes in TBW (by Urine Method) between Test Days				
<i>Subject</i>	<i>Shortest Interval (days)</i>	<i>Δ Total Body Water (kg)</i>	<i>Longest Interval (days)</i>	<i>Δ Total Body Water (kg)</i>
1	9	0.33	37	13.62
2	8	4.70	34	0.06
3	8	11.60	35	10.59
4	8	1.73	39	6.02
5	12	6.68	48	17.99
6	10	0.72	36	4.51
7	10	27.56	44	1.45
8	8	8.17	40	2.05
9	8	0.06	35	4.21
10	8	8.70	22	2.16
<i>Mean±SE</i>		<i>7.02±2.61</i>		<i>6.26±1.87</i>

These data demonstrate at least as much variability in the data collected at relatively short temporal intervals as over the longer intervals. The smaller differences seen between measurements of TBW taken 22-48 days apart than between those taken 8-12 days apart is interesting, but not significant ($p = 0.36$, $F = 1.115$, $df = 3, 27$).

In an attempt to further characterize sources of variance within the data, Figure 1 shows a plot of the subjects' initial body mass measurement (immediately prior to Trial A) vs. variance in the means from Trials A-D. For the urine determination method, where pooled variance was greater, the degree of variance appeared to be individual-dependent; in fact, the variance in the urine method increased with the size of the volunteer. A Pearson correlation analysis between subjects' characteristics and degree of variance using the urine method was carried out, and shown in Table 8.

Table 8. Subject Characteristics vs. Variance (Urine Method)		
Characteristic	<i>vs. Standard Deviation</i>	<i>vs. Variance</i>
Age (Years)	0.317	0.372
% Body Fat	0.742	0.655
LBM	0.813	0.811
Mean TBW	0.768	0.666
Total BM	0.861	0.831

Because variance was smaller using the plasma method, only the plasma values for each subject were compared with densitometry data. Mean values for TBW correlated with densitometry-determined LBM ($r = 0.866$). Lean body mass calculated from the initial body density for each of Trials A-D also correlated with TBW on each of those trials ($r = 0.938, 0.664, 0.684, 0.796$, respectively). These correlation coefficients place confidence at the 90-96% levels.

Linear regression using LBM (from body density) and individual daily weight as the independent variables, and TBW in Trials A-D as the dependent variables, reveals the best-fit regression line with a slope (\pm standard error) of 0.675 ± 0.096 and y-intercept of 8.482 ± 6.396 . Because doubling the error for the y-intercept gives a confidence interval that subsumes the origin, it must be assumed that this parameter should be dropped. Extrapolating the y-intercept to (0,0) gives a regression line with a slope of 0.801 ± 0.008 . Determining a similar regression line for initial LBM measurements made at the time of densitometry against mean values for TBW determined for each of the 10 subjects, once again gives a non-significant y-intercept. The regression line determined through the origin for the latter values gives a slope equal to 0.809 ± 0.012 . The mean values are plotted in Figure 2.

SUMMARY

Fat mass (FM) can be defined as that portion of the body's mass that is comprised exclusively of lipid. The remainder of the body mass is treated as essentially free of lipid (though this is obviously not the case in nature). The other body compartment, the “lean” compartment, has alternatively been referred to as the fat-free body mass (FFBM), the fat-free body weight (FFBW), fat-free wet weight (FFWW), or the lean body mass (LBM). Behnke's original concept of the LBM was more specific in its stated relationship to actively metabolizing tissue (1). It is assumed here that the body can be separated into these two compartments exclusively, and that the definitions for the lean compartment can be used interchangeably. However, it is recognized that the LBM does contain lipid and that the FM maintains some degree of hydration and metabolic activity.

Determination of body composition by underwater weighing is rapid and is precise to within approximately 1%, assuming no error in weight measurement (5). This estimate of precision assumes a 70 kg reference man has 19.3% body fat. Other assumptions in determining body density by this technique are that all "fat-free" body tissues are of equivalent density and equally hydrated, and that adipose tissue is equally dense throughout and essentially anhydrous. There are 5 factors most likely to contribute to errors in precision: differences in pulmonary residual volume, differences in gastrointestinal gas volume, differences in degree of tissue hydration, and intra-individual variation in FM. Residual volume is usually measured directly, where possible. Gastrointestinal (GI) gas volume is assumed to be insignificant using the present approach (3), and a source of negligible error. Other approaches account for a fixed,

small amount of GI gas volume, thus adding a constant correction factor (5). Correction for buoyancy in air during weighing is typically neglected, but is likely to cause error on the order of 0.2% or less.

While only a single determination of body density was performed in the present study, the low variance in total BM across the 4 trials, coupled with strict adherence to dietary and hydrational control, probably justify the assumption of minimal changes in body composition throughout the trials. Inaccuracy in densitometry estimations of LBM likely reflect inter-individual differences in composition of LBM (e.g., proportions of fat-free muscle mass, fat-free adipose mass, and fat-free bone mass); differences in the densities of these tissues; and differences in hydrational equilibrium state of each of these tissues (4). The accuracy of densitometry cannot be established without a suitable "gold standard" against which to normalize (Indirect and direct measures of body composition are impossible in the same living subject). Technical aspects of densitometry are fairly complex, requiring experienced personnel and accurately calibrated equipment. The test requires complete immersion of the subject. These factors make densitometry less than ideally suited for field studies. When safety, flexibility, and precision are considered, this may still be the most reliable index of body composition currently available. Newer techniques are promising (5,18).

Measurement of TBW by D₂O dilution is rapid, simple, and non-invasive (though small animal studies have shown mental status and metabolic changes with levels >10% (15)). The portability and the relative non-invasiveness of sampling make the method ideally suited for field studies. Sampling in plasma, urine, and saliva have been performed with equivalent ease

and efficacy, though salivary sampling may be less precise (13). Determination of body composition with this method relies on the same assumptions stated above for body density, with the caveat that deuterium-hydrogen ion exchange in the body will cause the method to overestimate TBW by a quantifiable amount. Precision estimates for D₂O dilution techniques have been cited to be as modest as 1-2% (5,15). However, these values actually reflect the variation in quantification of deuterium in the specimens, rather than reproducibility of the technique in humans. Culebras and Moore (6) have eloquently determined the maximal overestimation of TBW from isotope exchange during isotope dilution to be 5.22% of the TBW, but did not report the error associated with their estimate. In the present study, the mean of the coefficients of variation was 4.6%. This value should be understood as applicable for plasma assayed at 2 h of equilibration in healthy males. Precision of the assay technique likely accounts for some part of this error.

Precision may be jeopardized by allowing insufficient time for isotope equilibration, as was seen with the urine sampling in our larger subjects. As Schoeller and colleagues found (15) obese subjects appear to require 3-4 h to equilibrate in the urine. Based on the experience in the present study, this caution should be extended to include larger subjects (probably those greater than 75 kg total body mass). Subjects should void their bladders and be weighed immediately before administering the isotope. They should not eat or drink anything during the equilibration period, as new water taken in will not completely equilibrate in body tissues before obtaining samples. A more labor-intensive approach addresses the non-equilibrium state, as Coward et al. point out (5). The technique used for the present study quantifies the TBW at the *beginning* of

the equilibration period. By the time sampling is performed, some of this water has already left the body in the urine. For short equilibration times, the difference in TBW should be negligible. However, urinary loss of body water will be more significant with longer equilibration times.

The higher variability seen in this study for the urine assay probably relates to insufficient (variable) isotope equilibration, which was more marked in the subjects with higher body mass. This appears to indicate that distribution of deuterium is incomplete in the larger subjects, though differences in gastrointestinal absorption, renal excretion, or insensible losses cannot be excluded by this analysis. Schoeller and colleagues (15) found delayed isotope equilibration in the urine of obese subjects whom they studied. None of the subjects in the present study met criteria for obesity. In our subjects, body fat percentage correlated slightly less well with variance for the urine method than did total body mass. Both body fat content and body size are likely to affect distribution and therefore isotope equilibration. If the urine assay method is to be used, longer equilibration is recommended (3-4 h), particularly in larger subjects.

Because of the magnitude of variation in precision of the D₂O dilution technique, quantifying small changes in hydration or body composition with this technique would be difficult, as those changes are likely to fall within the confidence margins of the analysis. Effects of exercise, fluid administration, changes in insensible losses (e.g., as would be expected while in a hyperbaric chamber), or pharmacologic intervention during the equilibration period are not addressed here, but are likely to contribute additional error to the measurement.

Figure 2 shows a plot of the LBM determined from the hydrostatic weight vs. the mean values of TBW measured from D₂O equilibrated in plasma. Linear regression showed a best-fit

slope equal to 0.801 ± 0.008 . This "constant" theoretically defines the proportion of LBM that is water mass. Because the TBW values in this analysis were determined by lowering the actual values for D₂O dilution space by 4%, a similar linear regression analysis was done to determine the slope of the line relating LBM to D₂O space. As the confidence interval of the y-intercept subsumed the origin, the intercept term was dropped, giving the slope 0.833 ± 0.009 . Values for the constant reported in the literature range from 0.712 (16) to 0.808 (11), perhaps most commonly cited as 0.732 (5). This constant should always be interpreted on the basis of the population studied and techniques being compared. It should not be touted as a natural constant. At best, this "constant" can facilitate extrapolating values from one type of test to another.

In attempting to directly convert from TBW to LBM, one must be appreciative of the constants used to calculate both TBW and LBM. Deuterium oxide dilution space measured by the technique outlined here should reliably correlate to values giving similar confidence when referencing LBM calculated by the formulae and techniques used in the present study, assuming that the population of interest is similar. Using constants derived from animal studies with extrapolation to human data, vice-versa, or across species is unwarranted (16). Using different isotope dilution methods, e.g., DHO, H₂¹⁸O, or tritium oxide (THO) will result in estimates for TBW that differ from those reported here, as different isotope exchange kinetics pertain. Using any of the many other constants available in the literature to determine body fat from density will give results dissimilar to those reported here.

It is hoped that this review of the precision and relative flexibility of each of these techniques will help guide future efforts in body composition analysis for diving and

decompression research.

REFERENCES

1. Behnke, A.R., "Physiological studies pertaining to deep sea diving and aviation, especially in relation to the fat content and composition of the body." Harvey Lecture 1941-1942, Vol. 37, p. 198.
2. Behnke, A.R., Feen, B.G., and Welham, W.C., "The specific gravity of healthy men. Body weight \div volume as an index of obesity." Journal of the American Medical Association, Vol. 118, pp. 495-8, 1942.
3. Brozek J., Grande, F., Anderson, J.T., and Keys, A., "Densitometric analysis of body composition: revision of some quantitative assumptions." Annals of the New York Academy of Sciences; Vol. 110, pp. 113-40, 1963.
4. Clarys, J.P., Martin, A.D., and Drinkwater, D.T., "Gross tissue weights in the human body by cadaver dissection." Human Biology, Vol. 56, No. 3, pp. 459-73, 1984.
5. Coward, W.A., Parkinson, S.A., and Murgatroyd, P.R., "Body composition measurements for nutrition research." Nutrition Research Review; Vol. 1, pp. 115-24, 1988.
6. Culebras, J.M. and Moore, F.D., "Total body water and the exchangeable hydrogen. I. Theoretical calculation of nonaqueous exchangeable hydrogen in man." American Journal of Physiology, Vol. 232, No.1, pp. R54-9, 1977.
7. Curley, M.D., Robin, G.J., and Thalmann, E.D., Percent body fat and human decompression sickness. Undersea Biomedical Research Vol. 16(Suppl.), p. 29, 1989.
8. Dembert, M.L., "Individual factors affecting decompression sickness." In: The Physiological Basis of Decompression: Proceedings of the 38th UHMS Workshop, Vann, R.D. (ed.) Undersea

- and Hyperbaric Medical Society, Bethesda, MD, Vol. 75 (Phys), pp. 355-367, 1989.
9. Gray, J.S., "Constitutional factors affecting susceptibility to decompression sickness." In: Decompression Sickness, Fulton, J.F.: W.B. Saunders Co. Ltd., Philadelphia, PA, pp. 182-91, 1951.
10. Lam, T.H. and Yau, K.P., "Analysis of some individual risk factors for decompression sickness in Hong Kong." Undersea Biomedical Research, 16(4):283-92, 1989.
11. Moore, F.D., Lister, J., Boyden, C.M., Ball, M.R., Sullivan, N., and Dagher, F.J., "The skeleton as a feature of body composition." Human Biology, Vol. 40, No. 2, pp. 135-88, 1968.
12. Murgatroyd, P.R. and Coward, W.A., "An improved method for estimating changes in whole-body fat and protein mass in man." British Journal of Nutrition, Vol. 62, pp. 311-4, 1989.
13. Pullicino, W.A., Coward, A., Stubbs, R.J., and Elia, M., "Bedside and field methods for assessing body composition: Comparison with the deuterium dilution technique." European Journal of Clinical Nutrition, Vol. 44, pp. 753-62, 1990.
14. Schilling, C.W., Carlston, C.B., and Mathias, R.A. (eds.), The physician's guide to diving medicine, (Undersea Medical Society) Plenum Press, New York, 1984.
15. Schoeller, D.A., van Santen, E., Peterson, D.W., Dietz, W., Jaspan, J., and Klein, P.D., "Total body water measurement in humans with ^{18}O and ^2H labeled water." American Journal of Clinical Nutrition, Vol. 33, pp. 2686-93, 1980.
16. Sheng, H.P. and Huggins, R.A., "A review of body composition studies with emphasis on total body water and fat." American Journal of Clinical Nutrition, Vol. 32, pp. 630-47, 1979.
17. Siri, W.S., "The gross composition of the body." In: Advances in biological and medical

physics, Lawrence, T.H. and Tobias, C.A., eds. Academic Press, New York, pp. 239-80, 1956.

18. Siwek, R.A., Wales, J.K., Swaminathan, R., Burkinshaw, L., and Oxby, C.B., “Body composition of fasting obese patients measured by in vivo neutron activation analysis and isotopic dilution.” Clinical Physiology and Physiological Measures, Vol. 8, No. 3, pp. 271-82, 1987.

19. Vann, R.D. and Thalmann, E.D., “Decompression physiology and practice.” In: The Physiology and Medicine of Diving, 4th edition, Bennett, P.B. and Elliot, D.H. (eds.), W.B. Saunders Co. Ltd., Philadelphia, p. 383, 1993.

20. Wagner, D.A. Measurement of body composition using deuterium oxide. Technical Paper 913, Acton, MA, Metabolic Solutions, Inc., 1990.

21. Wilmore, J.H., Vodak, P.A., Parr, R.B., Girandola, R.N., and Billing, J.E., “Further simplification of a method for determination of residual lung volume.” Medicine and Science in Sports and Exercise, Vol. 12, No. 3, pp. 216-8, 1980.

22. Wise, D.A., Constitutional factors in decompression sickness. NEDU 2-63, Panama City, FL, U.S. Navy Experimental Diving Unit, 1963.

23. Anonymous, “Medical Examinations.” Manual of the Medical Department, NAVMED P-117, MANMED 15-31, Washington, DC, U.S. Department of the Navy, Bureau of Medicine and Surgery, 1996.

24. U.S. Department of the Navy, Chief of Naval Operations (OPNAVINST 6110.1 Series), Washington, DC, 1994.

25. U.S. Department of the Navy, Chief of Naval Operations (NAVADMIN 148/94),
Washington, DC, 1994.